Biological Effects of Short-Term, High-Concentration Exposure to Methyl Isocyanate. VI. *In Vitro* and *In Vivo* Complement Activation Studies

by William P. Kolb,* Jay R. Savary,* Catherine M. Troup,† Darol E. Dodd,† and John D. Tamerius*

The ability of MIC to induce complement activation *in vitro* and *in vivo* was investigated. For the *in vitro* studies, both human and guinea pig serum or EDTA-plasma samples were exposed to 1167 to 1260 ppm MIC vapor for 15 min at room temperature. The human serum samples exposed to MIC showed significant reductions in Factor B, C2, C4, C3, C5, and total hemolytic complement CH_{50} activity levels. C6 functional activity was unaffected. The C3, C5, and CH_{50} functional activities in guinea pig serum (the only functional tests conducted on these samples) were more sensitive to MIC-mediated reduction than the corresponding activity reductions observed in the human serum samples. The human and single guinea pig EDTA-plasma samples exposed to MIC vapor showed no evidence of C3 consumption but did show significant reductions in CH_{50} levels. Thus, MIC vapor was able to activate, and thereby reduce serum complement C3 activity *in vitro* by a complement-dependent process. However, the data suggest at least one complement component other than C3 was inactivated in EDTA-plasma by a complement-independent mechanism.

For the *in vivo* studies, five pairs of guinea pigs were exposed to 644 to 702 ppm MIC vapor until one of the pair died (11-15 min). MIC exposure was then discontinued, the surviving guinea pig was sacrificed, and EDTA-plasma was obtained from both animals and analyzed for complement consumption. Clear evidence was obtained to indicate that complement activation had occurred in these animals exposed to MIC for 11 to 15 min. In addition, the complement activation profile observed in these guinea pigs was qualitatively similar to that seen in the guinea pig serum samples exposed to MIC vapor *in vitro*. The total protein concentration present in plasma samples obtained from guinea pigs that had died from MIC exposure was elevated significantly ($p < 0.05$) above the total protein concentration present in plasma samples obtained from these same animals before MIC exposure or from paired animals which had not died (sacrificed) from MIC exposure. The possible contribution of complement activation to the fatal reaction(s) observed in these MIC-treated animals is discussed.

Introduction

Complement (C) is a cascade system of circulating plasma proteins consisting of 20 different components that collectively represent approximately 10 to 15% of the globulin, i.e., nonalbumin fraction of normal human serum (1-4). In general, complement components are activated sequentially upon the conversion of an inactive zymogen to an active proteolytic enzyme capable of cleaving, and thereby activating the next component in the reaction sequence (1-4). Polypeptide fragments of complement proteins produced as a result of cleavage by complement enzymes, as well as the subcomponents of the C1 complex, are designated by a small letter, e.g., the fifth component of complement (C5) is fragmented to C5a and C5b polypeptides as a result of complement activation (5).

Complement activation can be triggered via two distinct and separate pathways termed the classical and alternative activation pathways (1-4). The complement proteins unique to the classical pathway are C1, a calcium-dependent complex of subcomponents C1q, C1r, and C1s (present in the C1 complex in molar ratios of 1:2:2 respectively), C4, and C2. Initiation of the classical pathway of complement activation is triggered upon binding of C1q either to IgG or IgM-immunoglobulin containing antigen-antibody complexes (immune complexes) or to a variety of biologically relevant activating substances, as detailed in a recent review by Cooper (6).

*Department of Complement Research, Cytotech, Inc., 11045 Roselle Street, San Diego, CA 92121.

†Bushy Run Research Center, R.D. 4, Mellon Road, Export, PA 15632.

Complement proteins which are unique to the alternative pathway of complement activation are Factors B, D, and P (properdin) (3). However, functional alternative pathway activation also requires the presence of the complement regulator proteins Factor H (B_1H) and Factor I (C3b inactivator) (7). Alternative pathway activation is triggered by IgA containing immune complexes and a variety of naturally occurring microbial and eukaryotic cellular products (3). Initiation of either complement activation pathway leads to the activation of the C3 through C9 components of complement resulting in assembly of the C5b-9 complex on the target surface (4).

Complement activation can result in the expression of at least three distinct, physiologically relevant biological activities: (1) opsonization and enhanced phagocytosis of complement-coated target cells or particles by phagocytic white cells [target particle-bound C3 and C4 activation fragments mediate this activity by binding to complement receptors present on the outer membrane surfaces of phagocytic white cells (8)]; (2) irreversible structural and functional damage to target membranes resulting in cytolysis mediated by the C5b-9 membrane attack complex (4); and (3) activation of specialized physiological as well as cellular functions. The C4a, C3a, and C5a anaphylatoxin activation peptides are the complement mediators of these physiological and cellular functions which can initiate or contribute to acute inflammatory responses. The complement anaphylatoxins have the ability to induce smooth muscle contraction, increase vascular permeability, and release histamine from mast cells (9). In addition to its anaphylatoxin activities, C5a is also a very potent mediator of lysosomal enzyme release and directed chemotaxis of polymorphonuclear neutrophils and monocytes (9,10).

The complement system, i.e., complement anaphylatoxins in particular, is very important in the mediation of acute inflammatory injury in the lung. For example, monkeys immunized against pigeon serum (11) or guinea pigs immunized against ovalbumin (12) develop acute hemorrhagic, exudative alveolitis within 2 hr following aerosol antigen challenge. The pulmonary inflammatory damage observed in these models of lung injury is suppressed if the animals are complement depleted with cobra venom factor prior to antigen challenge (12). Furthermore, several lines of evidence have implicated a major role for complement activation in the mediation and/or perpetuation of acute lung inflammatory injury observed in the adult respiratory distress syndrome (ARDS). Thus, a neutrophil-stimulating factor, apparently C5-derived, has been reported to be present in the plasma of ARDS patients (13). The lung injury associated with sepsis in animal models of ARDS, either in guinea pigs or mice, was found to be complement and neutrophil dependent (14). C5a, C5a des arg, and C5 activated by noncomplement proteases (C5′) produce acute inflammatory lung injury in experimental animals subsequent to intratracheal instillation, which morphologically resembles the lung injury observed in ARDS (15,16). In addition, a recent study by Stevens

et al. (17) that used a primate model of ARDS induced by acute E. coli sepsis demonstrated that IV perfusion of an antibody reactive with human C5a anaphylatoxin effectively protected these animals against severe lung injury.

Since MIC inhalation by experimental animals produces major structural and functional pulmonary abnormalities (18), the effects of MIC on the complement system were investigated both in vitro by using human or guinea pig serum and EDTA-plasma samples, as well as in vivo by using a guinea pig inhalation model (19).

Materials and Methods

In Vitro MIC Exposure Studies

Blood samples were collected from human volunteers by venipuncture into vacutainers (Becton-Dickinson, Co., Rutherford, NJ) for the preparation of serum (red-top tubes) or EDTA-plasma (lavender-top tubes). Three to five milliliters of EDTA-whole blood were centrifuged immediately after collection at $1200g$ for 20 min at 4°C in a refrigerated centrifuge. Seven to ten milliliters of whole blood collected in red-top vacutainers were clotted at 37°C for 20 min, the clot was reamed, and the vacutainer tubes were incubated at 37°C for an additional 30 min. The EDTA-plasma or serum samples were exposed to MIC or air alone immediately after preparation. After experimental exposure, as described below, all samples were immediately frozen at −70°C in 100- to 200-μL portions until analyzed. Portions of the EDTA-plasma or serum samples receiving no treatment whatsoever (untreated controls) were frozen immediately after preparation at −70°C in 100- to 200-μL portions until analyzed.

Samples were exposed to statically generated MIC vapor in an inhalation chamber (19) for 15 min at room temperature. Individual EDTA plasma or serum samples, 2 mL each, were placed into 30-mL beakers on a stir plate (Sybran Nuova 7 Stir Plate) with a magnetic stir bar operating at approximately 50 rpm. Sham-exposed control samples were exposed to air only in the inhalation chamber by the same procedure for 15 min at room temperature. After exposure, the samples were removed from the exposure chamber and placed on ice in the chamber glove box for approximately 30 min to ensure that all the MIC vapor had dissipated from the experimental specimens. All samples were then frozen at −70°C in 100- to 200-μL portions until analyzed.

Inhalation Studies

Specific pathogen-free, female Sprague-Dawley guinea pigs (Hazelton-Research Animals, Denver, PA) were exposed to a target MIC vapor concentration of 650 ppm as described by Dodd et al. (19). Five groups of two animals were exposed to a target concentration of 650 ppm MIC until one of the animals died. At this point, MIC exposure was discontinued and EDTA-blood was collected as quickly as possible from the abdominal

aorta of the guinea pig which died. EDTA-plasma was prepared as described above. Concomitantly, the surviving guinea pig was sacrificed using methoxyfluorane anesthetic and EDTA-plasma was collected as described above. All EDTA-plasma samples were frozen immediately in single-use aliquots (100 μL) and stored at −70°C until analyzed.

Complement Reagents and Test Procedures

CH50 (*20*), C3 (*21*), C5 (*22*), and C6 (*23*) quantitative hemolytic activity assays were conducted as described. C4 functional activity was quantitated by incubating 25 μL of the sample dilution to be tested with 10 μL C4-deficient guinea pig serum and 3×10^7 EA (antibody sensitized sheep erythrocytes) in a final volume of 50 μL GVB^{++} (5 mM barbital, 135 mM NaCl, 0.1% gelatin, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, pH 7.4) for 30 min at 37°C. C2 and Factor B functional activity levels were quantitated by using immunochemically depleted C2- or Factor B-depleted human serum obtained from Cytotech, Inc. (San Diego, CA) following their recommended procedure.

Concentrations of iC3b in the human serum and plasma samples were quantitated by using monoclonal antibody 013III-1 obtained from Cytotech, Inc. (San Diego, CA). This monoclonal antibody is specific for a neo-antigenic determinant which is expressed by the iC3b catabolic fragment of C3 activation, but is not expressed by native C3 and is not expressed by C3b, C3d,g or C3d fragments (*24*). The 013III-1 monoclonal antibody was employed in a quantitative enzyme immunoassay (EIA) in which the monoclonal antibody was coated onto polystyrene microtiter plates, 100 μL/well, at 5 μg monoclonal antibody/mL borate buffered saline (25 mM sodium borate, 100 mM boric acid, 75 mM NaCl, pH 8.3) for 16 to 24 hr at 4°C. Duplicate, 100 μL portions of diluted specimen samples were incubated with 013III-1 coated wells for 30 min at room temperature (22°C). The wells were washed three times with PBS plus 0.05% Tween 20 by flooding and decanting, and 100 μL of HRP-conjugated goat anti-human C3 were added per well for 30 min at 22°C. The wells were washed three times with PBS plus 0.05% Tween 20 by flooding and decanting. A 100 μL volume of 0.034% 2-2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) diammonium salt (ABTS) in 100 mM sodium citrate, pH 4.0, plus 0.0126% hydrogen peroxide was added per well for 30 min at 22°C. The HRP enzymatic reaction was stopped by the addition of 50 μL 0.01% sodium azide. All wells were read at 405 nm with an automated Titertek Multiskan MC plate reader, and the iC3b concentration was determined from a standard curve run with each set of test samples.

Protein Determinations

Protein determinations were performed by the Folin procedure of Lowry (*25*).

Statistics

A significant change ($p < 0.05$) in complement component activity subsequent to *in vitro* or *in vivo* MIC exposure was determined by a one-way analysis of variance on the experimental data points before normalization to sham-exposed control values. Where appropriate, results are reported as the mean ± one standard deviation (SD).

Results and Discussion

Four different normal human serum and matched EDTA-plasma samples were exposed to 1167 ppm MIC vapor or air alone (sham-exposed control) for 15 min. The results from this set of experiments are seen in Table 1. The human serum samples exposed to MIC vapor showed significant reductions in total hemolytic complement CH_{50} levels, as well as significant reductions in the specific functional activities of Factor B, C2, C4, C3, and C5. In addition, all MIC-treated samples exhibited significantly increased quantities of iC3b, a breakdown fragment of C3 activation, as compared to sham-exposed control samples. In fact, the serum CH_{50} and iC3b values correlated well with each other, i.e., the greater the reduction in CH_{50} activity, the greater the amount of iC3b produced. Serum C6 functional activity levels were unaffected by MIC exposure.

From these results (Table 1) it appears that both complement pathways had been activated in the human serum samples since Factor B, C2, and C4 levels were all reduced to approximately the same extent. Activation of both pathways was further supported by the observation that no iC3b was formed in the human EDTA-plasma samples upon exposure to MIC vapor. Therefore, the C3 activation event in serum appeared to be dependent upon the presence of divalent cations.

The effect of MIC exposure on the complement system in guinea pig serum or EDTA-plasma was also investigated. As seen in Table 2, the CH_{50} and C5 functional activities in guinea pig serum were more sensitive to inactivation, following a 15 min *in vitro* exposure to 1260 ppm MIC vapor, than the corresponding activities observed in the human serum samples (Table 1). However, the general profile of complement component consumption was qualitatively similar. Namely, of the three complement functional activities tested in the guinea pig serum samples, the C3 levels demonstrated the smallest activity reduction while the CH_{50} and C5 levels demonstrated much greater activity reductions.

The complement CH_{50} activity measured in the guinea pig EDTA-plasma sample (Table 2) appeared more sensitive to MIC-mediated consumption than the corresponding CH_{50} activity measured in the human EDTA-plasma sample (Table 1) (note $n = 1$). These results further indicated that in both human and guinea pig plasma exposed to MIC vapor, the reduction in CH_{50} activity was much greater than the reduction in C3 functional activity (in the case of the human plasma samples, an insignificant increase in iC3b levels subsequent to

Table 1. Consumption of complement activity in normal human serum and EDTA-plasma
following 15 min *in vitro* exposure to 1167 ppm MIC vapor.

| | | Consumption of complement activity | | | | | | | | | |
| | | Serum[a] | | | | | | | | Plasma[a] | |
Sample no.	Treatment	CH_{50}	Factor B	C2	C4	C3	iC3b	C5	C6	CH_{50}	iC3b
1	MIC	51.4[a]	24.6	65.2	75.0	81.8	980.0	22.2	104.1	—	100.0
2	MIC	65.9	40.6	54.5	46.7	73.1	360.0	16.2	86.3	—	100.0
3	MIC	42.3	31.2	70.7	50.0	76.2	4410.0	27.3	112.2	59.8	100.0
4	MIC	67.0	91.4	79.1	53.8	100.0	312.0	24.3	108.2	—	71.8
Average	MIC	56.7*	47.0*	67.4*	56.4*	82.8*	1515.5†	22.5*	102.7	59.8	93.0
SD		11.9	30.4	10.3	12.8	12.0	1953.5	4.7	11.4		14.1

[a] All numbers represent the percent of sham-exposed (0 ppm MIC) control values.
*Significantly different from the sham-exposed (0 ppm MIC) control values, $p < 0.05$.
†Significantly different from the sham-exposed (0 ppm MIC) control values, $p < 0.10$.

Table 2. Consumption of complement activity in normal guinea pig serum and
EDTA-plasma following 15 min *in vitro* exposure to 1260 ppm MIC vapor.

| | | Consumption of complement activity | | | | | |
| | | Serum[a] | | | Plasma[a] | | |
Sample no.	Treatment	CH_{50}	C3	C5	CH_{50}	C3	C5
1	MIC	0	30.9	15.6	—	—	—
2	MIC	0	22.0	0	—	—	—
3	MIC	0	11.5	0	—	—	—
4	MIC	—	—	—	0	78.5	36.2
Average	MIC	0*	21.5*	5.2*	0	78.5	36.2
SD		—	9.7	9.0			

[a] All numbers represent the percent of sham-exposed control values.
*Significantly different from the sham-exposed (0 ppm MIC) control values, $p < 0.05$.

MIC exposure was interpreted to indicate that C3 functional activity was unaffected). This observation strongly suggested that one or more of the classical pathway proteins was inactivated directly by MIC in the absence of divalent cations, i.e., in the presence of 10 mM EDTA. On the other hand, as indicated in both Tables 1 and 2, C3 consumption did occur in both human and guinea pig serum by a complement-dependent process. This conclusion is based upon the significant reduction in C3 activity observed in the human or guinea pig serum samples as compared to the EDTA-plasma samples after MIC vapor exposure.

Guinea pig serum and matched EDTA-plasma samples were also exposed to a target concentration of 225 ppm MIC vapor using the same experimental procedure as described above. The results from this study (data not shown) indicated that 225 ppm MIC vapor induced a complement consumption profile which was similar to that observed in Table 2, however, the extent of complement consumption was not as great. For example, three guinea pig serum samples exposed to 225 ppm MIC vapor *in vitro* demonstrated average CH_{50} and C5 values, when expressed as a percent of sham-treated controls, of 42% and 37%, respectively.

To investigate the *in vivo* effects of MIC inhalation, paired guinea pigs were exposed to a target MIC vapor concentration of 650 ppm until one of the pair died (11–15 min). The MIC exposure was discontinued, the second animal was sacrificed, and EDTA-plasma samples

were obtained from both animals as described in "Materials and Methods." The EDTA-plasma samples were assayed for CH_{50}, C3, and C5 complement activities and the results are presented in Tables 3 and 4 as activity units per mL as well as activity units per mg protein. As seen in Tables 3 and 4, complement activation had occurred during the short time period (approximately 11–15 min) these animals were exposed to MIC vapor. In addition, the complement activation profile observed in these animals was qualitatively similar to the complement activation profile seen in the guinea pig serum samples exposed to MIC vapor *in vitro* (Table 2). Namely, the CH_{50} values were reduced to a greater extent than the C3 or C5 activity levels, and the C5 activity levels were reduced more than the C3 activity levels.

The protein concentration of plasma samples obtained from guinea pigs which had died from MIC exposure (Table 3) were elevated significantly ($p < 0.05$) above the pre-exposed protein concentrations as determined by analysis of variance. In contrast, the protein concentrations of plasma samples obtained from the paired guinea pigs, which were sacrificed after MIC exposure, were not significantly different from the predose protein concentrations. Thus, as a result of these observed differences in plasma protein concentrations, the functional complement activity levels summarized in Tables 3 and 4 were normalized per milligram plasma protein. These protein normalized values provide the best data

Table 3. Complement parameters in guinea pigs found dead after exposure to 650 ppm MIC.

Animal no.	MIC, ppm	CH_{50}/mL	$C3H_{50}$/mL	$C5H_{50}$/mL	mg Protein/ mL	CH_{50}/mg[a]	$C3H_{50}$/ mg[a]	$C5H_{50}$/ mg[a]	% Pre-exposed		
									CH_{50}	$C3H_{50}$	$C5H_{50}$
288 pre-exposed		1390	16900	9140	58	24.0	291	158			
exposed	685	1170	17000	8330	67	17.5	254	124	72.9	87.3	78.4
293 pre-exposed		1750	18800	10900	56	31.3	336	195			
exposed	644	1790	23100	11800	72	24.9	321	164	79.6	95.5	84.1
299 pre-exposed		1430	17400	9580	57	25.1	305	168			
exposed	702	1310	21100	10300	68	19.3	310	151	76.9	101.6	89.9
301 pre-exposed		1490	16300	8890	57	26.1	286	156			
exposed	691	1510	19700	10700	70	21.6	281	153	82.8	98.3	98.1
304 pre-exposed		1320	15600	9820	51	25.9	306	193			
exposed	648	1080	17900	9640	65	16.6	275	148	64.1	89.9	76.7
Average ± SD											
pre-exposed	674 ± 26	1476 ± 165	17000 ± 1210	9666 ± 780	56 ± 3	26.5 ± 2.8	305 ± 20	174 ± 19			
exposed	674 ± 26	1372 ± 285	19760 ± 2451	10154 ± 1286	68* ± 3	20.0* ± 3.4	288 ± 27	148* ± 15	75.3* ± 7.2	94.6 ± 5.8	85.5* ± 8.8

[a] The per milligram protein normalization calculations were based on the Folin protein determination values using bovine serum albumin for the standard curve.
* Significantly different from pre-exposed values, $p < 0.05$.

Table 4. Complement parameters in guinea pigs sacrificed after exposure to 650 ppm MIC vapor.

Animal no.	MIC, ppm	CH_{50}/mL	$C3H_{50}$/mL	$C5H_{50}$/mL	mg Protein/ mL	CH_{50}/mg[a]	$C3H_{50}$/ mg[a]	$C5H_{50}$/ mg[a]	% Pre-exposed		
									CH_{50}	$C3H_{50}$	$C5H_{50}$
302 pre-exposed		1410	17000	9940	54	26.1	315	184			
exposed	685	864	14200	6900	50	17.3	284	138	66.3	90.2	75.0
296 pre-exposed		1360	15800	10000	57	23.9	277	175			
exposed	644	609	13900	7270	70	8.7	199	104	36.4	71.8	59.4
303 pre-exposed		1390	19000	9640	56	24.8	339	172			
exposed	702	745	16500	7690	54	13.8	306	142	55.6	90.3	82.6
298 pre-exposed		1730	19000	11800	53	32.6	358	223			
exposed	691	700	14200	5800	53	13.2	268	109	40.5	74.9	48.9
305 pre-exposed		1560	18300	11200	55	28.4	333	204			
exposed	648	1000	17200	9880	58	17.2	297	170	60.6	89.2	83.3
Average ± SD											
pre-exposed	674 ± 26	1490 ± 155	17820 ± 1394	10516 ± 933	55 ± 2	27.2 ± 3.5	324 ± 31	192 ± 22			
exposed	674 ± 26	784 ± 152	15200* ± 1531	7510* ± 1500	57 ± 8	14.0* ± 3.5	271 ± 43	133* ± 27	51.9* ± 13	83.3 ± 9	69.8* ± 15

[a] The per milligram protein normalization calculations based on the Folin protein determination values using bovine serum albumin for the standard curve.
* Significantly different from pre-exposed values, $p < 0.05$.

for comparative purposes since it appears that these animals have lost water from their circulatory systems with the retention of plasma proteins. This conclusion is supported by the $C3H_{50}$/mL data presented in Table 3. The average pre-exposure $C3H_{50}$ values in these animals was 17,000 units/mL. The average C3H50 values in the same animals after death was 19,760 units/mL. It is highly unlikely that the livers of these animals synthesized sufficient quantities of C3 in 11–15 min to increase functional C3 levels in their circulating plasma by 16%. The C3 levels in the plasma obtained from the sacrificed animals, which experienced the same MIC exposure, showed no corresponding increase. These observations suggest that guinea pigs that died upon MIC exposure experienced shock, or possibly an anaphylactoid reaction before death resulting in the loss of fluid, but not plasma proteins, from their circulatory system.

This conclusion is at least partially supported by the studies of Troup et al. (26), which reported a significant increase in plasma hematocrit levels observed in rats exposed to 1000 ppm MIC for periods of 1 hr or longer.

The MIC-induced activation of C5 in vivo, as evidenced by the results presented in Tables 3 and 4, may be a major contributor to the increased hematocrit (26), neutrophilia (26), and pulmonary perivascular neutrophil extravasation (18) observed in MIC-treated animals. This would be the case whether C5 activation occurred in these animals as a result of sequential, physiological complement pathway activation or as an isolated event as exemplified by the activation of C5 by noncomplement proteases. Thus, a variety of noncomplement enzymes, e.g., the neutrophil lysosomal proteases elastase (27) or cathepsin G (28), as well as the coagulation enzymes plasmin (29), and α-thrombin

(29,30), can hydrolyze C5 to form either a modified C5′ molecule or C5 fragments that express C5a-like biological activities (15,27–31). Either one or both C5 activation mechanism(s) could be operational in MIC-treated animals as a result of the rapid bronchiolar epithelial necrosis and sloughing, described by Fowler et al. (18), leading to the influx of plasma proteins into the major airways. The exposure of plasma complement to necrotic cells would result in classical pathway activation (32,33) and C5a anaphylatoxin production. Alternatively, proteolytic enzymes released from the necrotic epithelium, infiltrating neutrophils, or blood clots could lead to the activation of C5 directly (27–31).

Although these results suggest that guinea pigs which died subsequent to MIC vapor exposure experienced either shock or an anaphylactoid reaction, which may have contributed to the animals' death, the extent of complement activation was actually greater in the guinea pigs that did not die but were sacrificed. This can be seen clearly by comparing the average percent of pre-exposure CH_{50}/mg, $C3H_{50}$/mg, and $C5H_{50}$/mg values for the sacrificed animals (Table 4) with these same values obtained for the animals that died as a result of MIC exposure (Table 3). Taken singularly, these data suggest that intravascular complement activation may help delay the onset of death in these animals. Alternatively, the sacrificed animals may have been less susceptible to the deleterious effects of MIC-induced complement activation.

In conclusion, the *in vitro* exposure of human or guinea pig serum and matched EDTA-plasma samples or the *in vivo* exposure of guinea pigs to MIC vapor induced profound alterations in the complement system. These complement alterations resulted in reductions of several key complement component functional activities. However, the complement activity profiles observed in limited *in vitro* studies were very unusual and could not be explained fully on the basis of a normal, physiological activation event. Therefore, a complement-independent mechanism must be invoked, e.g., direct MIC chemical inactivation of one or more complement proteins, to explain the reductions in complement activity levels observed in the one human, and one guinea pig MIC-exposed EDTA-plasma sample.

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